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United States Patent [19][11] **Patent Number:** **5,908,626****Chang et al.**[45] **Date of Patent:** ***Jun. 1, 1999**[54] **HYBRID WITH INTERFERON- β AND AN IMMUNOGLOBULIN FC JOINED BY A PEPTIDE LINKER**[75] Inventors: **Tse Wen Chang**, Hsinchu, Taiwan;
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[*] Notice: This patent is subject to a terminal disclaimer.

[21] Appl. No.: **08/994,719**[22] Filed: **Dec. 19, 1997****Related U.S. Application Data**

[63] Continuation-in-part of application No. 08/719,331, Sep. 25, 1996, Pat. No. 5,723,125, which is a continuation-in-part of application No. 08/579,211, Dec. 28, 1995, abandoned.

[51] **Int. Cl.**⁶ **A61K 39/395**; A61K 39/00;
C07K 1/00; C07K 16/00[52] **U.S. Cl.** **424/134.1**; 424/185.1;
424/192.1; 435/69.7; 530/387.3; 530/351[58] **Field of Search** 530/387.3, 351;
424/134.1, 185.1, 192.1; 435/69.7; 536/23.4[56] **References Cited****U.S. PATENT DOCUMENTS**

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[57] **ABSTRACT**

Disclosed is a hybrid recombinant protein consisting of human interferon- β , and a human immunoglobulin Fc fragment, preferably $\gamma 4$ chain, joined by a peptide linker comprising the sequence Gly Gly Ser Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser (SEQ ID NO:1).

1 Claim, No Drawings

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HYBRID WITH INTERFERON- β AND AN IMMUNOGLOBULIN FC JOINED BY A PEPTIDE LINKER

This application is a continuation-in-part of U.S. application Ser. No. 08/719,331, filed Sep. 25, 1996, now U.S. Pat. No. 5,723,125 which is a continuation-in-part of U.S. application Ser. No. 08/579,211, filed Dec. 28, 1995, now abandoned.

BACKGROUND OF THE INVENTION

Interferons, including interferon- α ("IFN α ") and interferon- β ("IFN β "), were among the first of the cytokines to be produced by recombinant DNA technology. IFN α has been shown to have therapeutic value in conditions such as inflammatory, viral, and malignant diseases. IFN β has been approved for use in treatment of multiple sclerosis.

Most cytokines, including IFN β , have relatively short circulation half-lives since they are produced in vivo to act locally and transiently. To use IFN β as an effective systemic therapeutic, one needs relatively large doses and frequent administrations. Such frequent parenteral administrations are inconvenient and painful. Further, toxic side effects are associated with IFN β administration which are so severe that some multiple sclerosis patients cannot tolerate the treatment. These side effects are probably associated with administration of a high dosage.

To overcome these disadvantages, one can modify the molecule to increase its circulation half-life or change the drug's formulation to extend its release time. The dosage and administration frequency can then be reduced while increasing the efficacy. With respect to interferon- α , which suffers from the same disadvantages, efforts have been made to create a recombinant IFN α -gelatin conjugate with an extended retention time (Tabata, Y. et al., *Cancer Res.* 51:5532-8, 1991). A lipid-based encapsulated IFN α formulation has also been tested in animals and achieved an extended release of the protein in the peritoneum (Bonetti, A. and Kim, S. *Cancer Chemother Pharmacol.* 33:258-261, 1993).

Immunoglobulins of IgG and IgM class are among the most abundant proteins in the human blood. They circulate with half-lives ranging from several days to 21 days. IgG has been found to increase the half-lives of several ligand binding proteins (receptors) when used to form recombinant hybrids, including the soluble CD4 molecule, LHR, and the IFN- γ receptor (Mordenti J. et al., *Nature*, 337:525-31, 1989, Capon, D. J. and Lasky, L. A., U.S. Pat. No. 5,116,964; Kurschner, C. et al., *J. Immunol.* 149:4096-4100, 1992). However, such hybrids can present problems in that the peptide at the C-terminal of the active moiety and the peptide at the N-terminal of the Fc portion at the fusion point creates a new peptide sequence, which is a neoantigen, and which can be immunogenic. The invention relates to a IFN β -Fc hybrid which is designed to overcome this problem and extend the half-life of the IFN β .

SUMMARY OF THE INVENTION

The present invention relates to a hybrid recombinant protein which consists of two subunits. Each subunit includes a human interferon, preferably IFN β , joined by a peptide linker which is primarily composed of a T cell inert sequence, linked to a human immunoglobulin Fc fragment, preferably the γ 4 chain. The γ 4 chain is preferred over the γ 1 chain because the former has little or no complement activating ability.

The C-terminal end of the IFN β is linked to the N-terminal end of the Fc fragment. An additional IFN β (or other cytokine) can attach to the N-terminal end of any other unbound Fc chains in the Fc fragment, resulting in a homodimer for the γ 4 chain. If the Fc fragment selected is another chain, such as the μ chain, then, because the Fc fragments form pentamers with ten possible binding sites, this results in a molecule with interferon, or another cytokine, linked at each of ten binding sites.

The two moieties of the hybrid are linked through a T cell immunologically inert peptide (e.g., Gly Gly Ser Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser (SEQ ID NO:1)). This peptide itself is immunologically inactive. The insertion of this peptide at the fusion point eliminates the neoantigenicity created by the joining of the two peptide moieties. The linker peptide also increases the flexibility of these moieties and allows retention of the biological activity. This relatively long linker peptide helps overcome the possible steric hindrance from the Fc portion of the hybrid, which could interfere with the activity of the hybrid.

The hybrid should have a much longer half-life than the native IFN β , based on experiments with a similar hybrid but in which IFN α is the cytokine moiety. Due to the linker, the hybrid is also designed to reduce the possibility of generating a new immunogenic epitope (a neoantigen) at what would otherwise be the fusion point of the IFN β and the immunoglobulin Fc segment.

Cytokines are generally small proteins with relatively short half-lives which dissipate rapidly among various tissues, including at undesired sites. It is believed that small quantities of some cytokines can cross the blood-brain barrier and enter the central nervous system, thereby causing severe neurological toxicity. The IFN β linked to Fc γ of the present invention would be especially suitable for treating multiple sclerosis, because these products will have a long retention time in the vasculature (upon intravenous administration) and will not penetrate undesired sites.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

SEQ ID NO:1 is the unique peptide linker which conjugates the N-terminal end(s) of a heavy chain γ 4 Fc fragment to an interferon- β moiety.

SEQ ID NO:2 is the nucleotide and amino acid sequence of interferon- β .

SEQ ID NO:3 is the nucleotide and amino acid sequence of interferon- β , the unique peptide linker, and an Fc immunoglobulin moiety.

DETAILED DESCRIPTION OF MAKING AND USING THE INVENTION

The hybrid molecule of the invention includes an interferon- β moiety linked through a unique linker to an immunoglobulin Fc moiety. Preferably, the C-terminal ends of two interferon moieties are separately attached to each of the two N-terminal ends of a heavy chain γ 4 Fc fragment, resulting in a homodimer structure. A unique linker peptide, Gly Gly Ser Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser (SEQ ID NO:1), was created to link the two moieties. The complete nucleotide sequence of the preferred hybrid (including the linker and the Fc moiety) appears in SEQ ID NO: 3. The linker is located at amino acid residue numbers 188 to 203.

The advantage of the hybrid over the native cytokine is that the half-life in vivo is longer. The hybrid including interferon and the γ 4 chain Fc homodimer is larger than the

native interferon. Because the pores in the blood vessels of the liver are large, this larger molecule is more suitable for use in treating hepatitis, where the virus responsible primarily affects the liver.

The linker peptide is designed to increase the flexibility of the two moieties and thus maintain their biological activity. Although the interferon and the immunoglobulin are both of human origin, there is always a possibility of generating a new immunogenic epitope at the fusion point of the two molecules. Therefore, the other advantage of the linker of the invention, which consists mainly of a T cell inert sequence, is to reduce immunogenicity at the fusion point. Referring to SEQ ID NO:3, it can be seen that if the linker (residue numbers 188 to 203) was not present, a new sequence consisting of the fusion point residues would be created. This new sequence would be a neoantigen for the human body.

IFN β is approved for use in treating multiple sclerosis. It may have other therapeutic uses as well. It is nearly as well studied and characterized as is interferon- α .

The advantages of the γ 4 chain as the Fc moiety in the hybrid is that it is stable in the human circulation. The γ 4 chain (unlike the γ 1 chain) also avoids the wide spectrum of secondary biological properties, such as complement fixation and antibody-dependant cell-mediated cytotoxicity (ADCC), which may be undesirable properties.

The cDNA of the IFN β can be obtained by reverse transcription and PCR, using RNA extracted from leukocytes which express IFN β , and following the extraction with reverse transcription and expression in a standard expression system.

There are several ways to express the recombinant protein in vitro, including in *E. coli*, baculovirus, yeast, mammalian cells or other expression systems. The prokaryotic system, *E. coli*, is not able to do post-translational modification, such as glycosylation. This could be a problem in these systems, and mammalian expression could be preferred for this reason.

There are also several other advantages to this mammalian expression system. First, the recombinant protein is secreted into the culture supernatant and there is no aggregation or inclusion bodies, thereby simplifying purification. One chromatography step using a protein A column yields a purified IFN α -Fc protein. Also, the protein produced in this system has a glycosylation pattern very similar to the natural molecules since it is expressed by mammalian cells.

As mentioned above, the purification of the IFN β -Fc recombinant protein from the culture supernatant is relatively straightforward. The protein with a purity of more than 90%, as judged by SDS-PAGE, can be easily obtained by one step of affinity chromatography with a protein A column.

There are several assay methods available for the measuring of the IFN β bioactivity, including an antiviral assay. The hybrid of SEQ ID NO:3 is expected to have a longer half-life in vivo than native IFN β based on results achieved using the same hybrid, but with interferon α as the cytokine. Even though its specific activity is lower, this novel hybrid is expected to be preferred to the native IFN β for clinical use. This is because, as a result of the longer half-life, the Cxt (the area under the concentration vs. time curve) would be up to several hundred times greater than for the native IFN β . This means that at the equivalent molar dosage of the native IFN β and the hybrid, the latter would provide a several hundred fold increased exposure to IFN β , resulting in vastly increased efficacy at the same dosage, and less frequent administration.

It should be understood that the terms and expressions used herein are exemplary only and not limiting, and that the scope of the invention is defined only in the claims which follow, and includes all equivalents of the subject matter of those claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 3

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 48 nucleic acids
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: double stranded
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGT GGC TCA GGT GGA TCC GGT GGA GGC GGA AGC GGC	36			
Gly Gly Ser Gly Gly Ser Gly Gly Gly Gly Ser Gly				
<table style="width: 100%; border-collapse: collapse;"> <tr> <td style="text-align: center;">1</td> <td style="text-align: center;">5</td> <td style="text-align: center;">10</td> </tr> </table>	1	5	10	
1	5	10		
GGT GGA GGA TCA	48			
Gly Gly Gly Ser				
<table style="width: 100%; border-collapse: collapse;"> <tr> <td style="text-align: center;">15</td> </tr> </table>	15			
15				

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 561 nucleic acids
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double stranded

-continued

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATG ACC AAC AAG TGT CTC CTC CAA ATT GCT CTC CTG TTG TGC TTC	45
Met Thr Asn Lys Cys Leu Leu Gln Ile Ala Leu Leu Leu Cys Phe	
1 5 10 15	
TCC ACT ACA GCT CTT TCC ATG AGC TAC AAC TTG CTT GGA TTC CTA	90
Ser Thr Thr Ala Leu Ser Met Ser Tyr Asn Leu Leu Gly Phe Leu	
20 25 30	
CAA AGA AGC AGC AAT TTT CAG TGT CAG AAG CTC CTG TGG CAA TTG	135
Gln Arg Ser Ser Asn Phe Gln Cys Gln Lys Gln Leu Asn Gly Arg	
35 40 45	
AAT GGG AGG CTT GAA TAC TGC CTC AAG GAC AGG ATG AAC TTT GAC	180
Leu Leu Trp Leu Glu Tyr Cys Leu Lys Asp Arg Met Asn Phe Asp	
50 55 60	
ATC CCT GAG GAG ATT AAG CAG CTG CAG CAG TTC CAG AAG GAG GAC	225
Ile Pro Glu Glu Ile Lys Gln Leu Gln Gln Phe Gln Lys Glu Leu	
65 70 75	
GCC GCA TTG ACC ATC TAT GAG ATG CTC CAG AAC ATC TTT GCT ATT	270
Thr Ile Asp Ala Ala Tyr Glu Met Leu Gln Asn Ile Phe Ala Ile	
80 85 90	
TTC AGA CAA GAT TCA TCT AGC ACT GGC TGG AAT GAG ACT ATT GTT	315
Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn Glu Thr Ile Val	
95 100 105	
GAG AAC CTC CTG GCT AAT GTC TAT CAT CAG ATA AAC CAT CTG AAG	360
Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn His Leu Lys	
110 115 120	
ACA GTC CTG GAA GAA AAA CTG GAG AAA GAA GAT TTC ACC AGG ATG	405
Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr Arg Met	
125 130 140	
AGC AGT GGA AAA CTC CTG CAC CTG AAA AGA TAT TAT GGG AGG ATT	450
Ser Ser Gly Lys Leu Leu His Leu Lys Arg Tyr Tyr Gly Arg Ile	
145 150 155	
CTG CAT TAC CTG AAG GCC AAG GAG TAC AGT CAC TGT GCC TGG ACC	495
Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr	
160 165 170	
ATA GTC AGA GTG GAA ATC CTA AGG AAC TTT TAC TTC ATT AAC AGA	540
Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg	
175 180 185	
CTT ACA GGT TAC CTC CGA AAC	561
Leu Thr Gly Tyr Leu Arg Asn	
190	

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1299 nucleic acids
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double stranded
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATG ACC AAC AAG TGT CTC CTC CAA ATT GCT CTC CTG TTG TGC TTC	45
Met Thr Asn Lys Cys Leu Leu Gln Ile Ala Leu Leu Leu Cys Phe	
1 5 10 15	
TCC ACT ACA GCT CTT TCC ATG AGC TAC AAC TTG CTT GGA TTC CTA	90
Ser Thr Thr Ala Leu Ser Met Ser Tyr Asn Leu Leu Gly Phe Leu	
20 25 30	
CAA AGA AGC AGC AAT TTT CAG TGT CAG AAG CTC CTG TGG CAA TTG	135
Gln Arg Ser Ser Asn Phe Gln Cys Gln Lys Gln Leu Asn Gly Arg	
35 40 45	
AAT GGG AGG CTT GAA TAC TGC CTC AAG GAC AGG ATG AAC TTT GAC	180

-continued

Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	
			325					330					
CTG	CCC	CCA	TCC	CAG	GAG	GAG	ATG	ACC	AAG	AAC	CAG	GTC	1044
Leu	Pro	Pro	Ser	Gln	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	
335					340						345		
AGC	CTG	ACC	TGC	CTG	GTC	AAA	GGC	TTC	TAC	CCC	AGC	GAC	1083
Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	
		350					355					360	
ATC	GCC	GTG	GAG	TGG	GAG	AGC	AAT	GGG	CAG	CCG	GAG	AAC	1122
Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	
				365					370				
AAC	TAC	AAG	ACC	ACG	CCT	CCC	GTG	CTG	GAC	TCC	GAC	GGC	1161
Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	
		375					380					385	
TCC	TTC	TTC	CTC	TAC	AGC	AGG	CTA	ACC	GTG	GAC	AAG	AGC	1200
Ser	Phe	Phe	Lys	Tyr	Ser	Arg	Leu	Thr	Val	Asp	Lys	Ser	
							390					395	
AGG	TGG	CAG	GAG	GGG	AAT	GTC	TTC	TCA	TGC	TCC	GTG	ATG	1239
Arg	Trp	Gln	Glu	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	
	400					405					410		
CAT	GAG	GCT	CTG	CAC	AAC	CAC	TAC	ACA	CAG	AAG	AGC	CTC	1278
His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	
			415					420					
TCC	CTG	TCT	CTG	GGT	AAA	TAG							1299
Ser	Leu	Ser	Leu	Gly	Lys								
425					430								

What is claimed is:

1. A hybrid molecule comprising an interferon- β molecule joined at its C-terminal end through a peptide linker to the N-terminal end of an immunoglobulin γ 4 chain Fc fragment,

the peptide linker comprising the sequence Gly Gly Ser Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser (SEQ ID NO:1).

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